Phosphate transporters of the SLC20 and SLC34 families

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Phosphate transporters of the SLC20 and SLC34 families

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Contents

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Abstract

Transport of inorganic phosphate (P_i) across the plasma membrane is essential for normal cellular function. Members of two families of SLC proteins (SLC20 and SLC34) act as Na^+-dependent, secondary-active cotransporters to transport P_i across cell membranes. The SLC34 proteins are expressed in specific organs important for P_i homeostasis: NaPi-IIa (SLC34A1) and NaPi-IIc (SLC34A3) fulfill essential roles in P_i reabsorption in the kidney proximal tubule and NaPi-IIb (SLC34A2) mediates P_i absorption in the gut. The SLC20 proteins, PiT-1 (SLC20A1), PiT-2 (SLC20A2) are expressed ubiquitously in all tissues and although generally considered as "housekeeping" transport proteins, the discovery of tissue-specific activity,regulatory pathways and gene-related pathophysiologies, is redefining their importance. This review summarises our current knowledge of SLC20 and SLC34 proteins in terms of their basic molecular characteristics, physiological roles, known pathophysiologies and pharmacology.

1. Introduction

In mammals, phosphorus is an essential element for cellular signaling, metabolic and synthetic pathways as well as fulfilling a necessary structural role in bone and phospholipid membranes. It is obtained from the diet as anionic inorganic phosphate (P_i) and requires active transport to traverse the cell membrane. Moreover, transepithelial transport of P_i in different epithelia (specifically in kidney and small intestine) contributes fundamentally to overall P_i homeostasis (Fig 1A). This is particularly evident in kidney where P_i reabsorption in the proximal tubule is regulated by a number of hormones and metabolic factors thereby adusting excretion of P_i according to the organism's P_i requirements. Any defects in the regulation of cotransporter proteins or the expression of mutated proteins can lead to severe pathophysiological conditions. Two genetically distinct families of sodium-coupled cotransporters mediate transport of P_i in mammals: SLC20 comprising SLC20A1 (PiT1), SLC20A2 (PiT2) and SLC34 comprising SLC34A1 (NaPi-IIa), SLC34A2 (NaPi-IIb) and SLC34A3 (NaPi-IIc) (see Tables 1, 2). Whereas the physiological roles of SLC34 proteins, with their well-defined tissue distribution, has been extensively investigated and characterized, the novel roles of the more ubiquitously expressed SLC20 proteins are only beginning to emerge in addition to that to which they were originally ascribed, as housekeeping proteins.

2. The SLC34 family (type II Na^+-coupled P_i transporters: NaPi-IIa, NaPi-IIb, NaPi-IIc)

2.1 NaPi-IIa

NaPi-IIa (SLC34A1) was identified by functional expression cloning using *X. laevis* oocytes and rat and human kidney cDNA library (Magagnin et al., 1993). The major site of expression is the renal proximal tubule where the 80-90kDa protein is localized in the microvilli that form the brush border membrane (BBM). In human tissue, significant mRNA expression is detected only in the kidney (Fig 1B) (Nishimura and Naito, 2008). Under normal physiological conditions, the abundance of NaPi-IIa is highest in the S1 proximal tubule segments of juxtamedullary nephrons (Custer M, 1994).

2.2 NaPi-IIb

NaPi-IIb (SLC34A2) was identified from a mouse embryo EST clone and was specifically localized to enterocytes of the small intestine (Hilfiker et al., 1998). In humans mRNA is detected in lungs, testis, salivary gland, thyroid gland, small intestine, liver, mammary gland, uterus but not in renal tissue (Fig 1B) (Nishimura and Naito, 2008).

2.3 NaPi-IIc

NaPi-IIc (SLC34A3) was identified from human EST clones (Segawa et al., 2002). Expression of NaPi-IIc was found exclusively in the kidney and proposed to be growth related based on animal studies: in weaning rats it is expressed in the BBM of S1 segments of proximal tubules, but is strongly down-regulated in adult animals (Miyamoto et al.; Segawa et al., 2002). The 75kDa protein is localized at the apical membranes of proximal tubule epithelia of juxtamedullary nephrons (Segawa et al., 2002). Like NaPi-IIa, the distribution of mRNA in human tissue is also renal specific (Fig 1B) (Nishimura and Naito, 2008).

2.4 Kinetics and structure-function relationships of SLC34 proteins

2.4.1 Kinetics of SLC34 proteins

All 3 isoforms preferentially transport divalent P_i (Bacconi et al., 2005; Forster et al., 1999) and are exclusively Na^+-dependent, although Li^+ ions (Andrini et al.) can partly replace Na^+ to drive transport. The transport capacity of all SLC34 proteins is strongly dependent on pH, which defines the monovalent/divalent P_i distribution in the extracellular compartment and protons also directly modulate the transport kinetics e.g.(Forster et al., 2000). At pH 7.4, the 3 isoforms display similar apparent substrate affinities of approximately 100 μM and 40 mM for divalent P_i and Na^+ respectively. Functionally, they can be distinguished as electrogenic (NaPi-IIa,b) and electroneutral (NaPi-IIc) cotransporters (Fig 2A). NaPi-IIa/b translocate one net positive charge per transport cycle and their transport rate is a function of membrane potential. They mediate transport with a 3:1 Na^+:P_i stoichiometry (Forster et al., 1999). The transport kinetics of NaPi-IIc are insensitive to membrane potential, no net charge is translocated and transport is mediated with a 2:1 stoichiometry (Bacconi et al., 2005; Segawa et al., 2002). The stoichiometry difference means that the theoretical P_i concentrating capacity is approximately 100-fold higher for NaPi-IIa/b, at the cost of a 10-fold greater inward flux of Na^+ ions compared to NaPi-IIc, together with net charge movement, both of which must be compensated by the cell through the action of the Na^+/K^+-ATPase.

Kinetic studies have established that for NaPi-IIa/b, 2 Na^+ ions bind sequentially and cooperatively before Pi (Virkki et al., 2006). A 3rd Na^+ binding transition precedes a rate-limiting reorientation of the fully loaded carrier. Li^+ ions can compete for occupancy of the the 1st Na^+ binding site (Andrini et al.).The order of release of substrates to the cytosol is unknown. For NaPi-IIc, the 1st Na^+, which confers electrogenicity to NaPi-IIa/b, still interacts but is not cotransported (Ghezzi et al., 2009). Electrogenic SLC34 proteins exhibit an uncoupled leak that is most likely mediated by Na^+ ions and is active only in the absence of P_i (Andrini et al., 2008). The Na^+-leak is not found for the electroneutral NaPi-IIc (Bacconi et al., 2005), nevertheless, naturally occurring mutations in NaPi-IIc have been reported to result in a significant Na^+-leak (Jaureguiberry et al., 2008).

2.4.2 Structure-function relationships of SLC34 proteins.

SLC34 proteins belong to a unique class of membrane proteins and share no homology with the other main transporter families, even at the bacterial level. The current lack of a 3-D structure of the mammalian SLC34 proteins or their bacterial homologs, means that structural information is based on indirect biophysical and biochemical studies on wild-type and engineered mutations, e.g.(Forster et al., 2002; Virkki et al., 2007). SLC34 proteins are all assumed to be functional monomers (Kohler et al., 2000), although indirect evidence suggests they may exist as dimers at the BBM (Forster et al., 2006; Gisler et al., 2007). The primary sequences of the mammalian isoforms vary from 599 amino acids (NaPi-IIc) to approximately 640 amino acids (NaPi-IIa and NaPi-IIb), with differences mainly in the intracellular C-and N-terminal regions and the large extracellular linker region. This linker contains 2 N-glycosylation sites and a disulfide bridge that links the two halves of the protein (Fig 2A).The currently proposed topology of the eukaryotic isoforms comprises 12 transmembrane domains (TMDs) (Fig 2). This model incorporates predictions from biochemical and biophysical studies including epitope labeling, cysteine scanning mutagenesis and *in vitro* glycosylation assays (for review see: (Forster et al.; Forster et al., 2006; Forster et al., 2002; Virkki et al.,

2007). The C-terminal region is important for targeting, hormonal regulation and protein-protein interactions, for example the TRL motif in the C-terminal plays a role as a PDZ binding motif (Hernando et al., 2001; Karim-Jimenez et al., 2001), and a KR motif located in an intracellular linker region (Fig 2A) is critical for PTH sensitivity (Karim-Jimenez et al., 2000). Sequence analysis reveals an inverted repeat motif that is conserved amongst SLC34 isoforms and homologs in all phyla (Werner and Kinne, 2001). The repeat regions contain two opposed reentrant loops formed by pairs of discontinuous helices linked by a short peptide stretch; studies confirm their functional role in defining the transport (Forster et al., 2002). Similar structural motifs are also found in the 3-D structures of transporters with known structure, e.g.(Forrest et al.; Screpanti and Hunte, 2007), which underscore their role in substrate coordination. All SLC34 proteins, including bacterial homologs, are expected to have similar functional core elements comprising TMDs2-10 (Fig. 2A). No information is available concerning the localization of the substrate coordination sites. The electroneutral NaPi-IIc (Segawa et al., 2002) provided an important tool to identify the molecular determinants of electrogenicity; in particular 3 critical amino acids essential for Na^+ binding and charge translocation, conserved in all electrogenic isoforms, were identified by mutagenesis (Bacconi et al., 2005).

2.5 Physiological, pathophysiological and pharmaceutical aspects of SLC34 proteins

2.5.1 Physiological role and regulation

The expression of NaPi-IIa and NaPi-IIc is limited to the BBM of renal proximal tubules (Custer M, 1994; Segawa et al., 2002). Both transporters together with PiT-2 (see 3.3.1) are responsible for the reabsorption of most of the P_i from the primary filtrate. However, their quantitative contribution is an open issue (e.g.(Villa-Bellosta and Sorribas)). NaPi-IIa seems to be the major transporter in murine kidneys, as mice deficient for this cotransporter are hypophosphatemic due to a major loss of P_i in urine (Beck et al., 1998). In contrast, urinary excretion and circulating levels of P_i are normal in NaPi-IIc$^{-/-}$ animals (Segawa et al., 2009). Based on these findings it was assumed that NaPi-IIa has a major quantitative impact on renal reabsorption of P_i. However, recent data suggest that NaPi-IIc may play more important role in humans (see 2.5.2) (Bergwitz et al., 2006; Miyamoto et al.).

NaPi-IIb has a wider pattern of expression (Hilfiker et al., 1998; Nishimura and Naito, 2008) and its constitutive depletion in mice is lethal (Shibasaki et al., 2009). It is the only SLC34 member present in intestinal microvilli. In mice, it is specifically located in the ileum (Radanovic et al., 2005), which is the intestinal segment of this species that exhibit a higher Na-dependent P_i cotransporter activity. Inducible depletion of NaPi-IIb in mice results in fecal loss of P_i, indicating that this protein is required for proper intestinal absorption of P_i (Sabbagh et al., 2009). However, the mutant mice are normophosphatemic due to a compensatory upregulation of NaPi-IIa that results in increased renal reabsorption.

The abundance of SLC34 proteins at the apical membrane of renal proximal tubules or intestinal microvilli is the main determinant of P_i transport capacity. Their abundance is controlled by metabolic factors (dietary P_i), vitamins (1,25 dihydroxy vitamin D3, $1,25(OH)_2D$) and hormones , including parathyroid hormone (PTH), dopamine, fibroblast growth factor 23 (FGF-23) and its co-receptor Klotho that regulate P_i homeostasis (for review see (Bergwitz and Juppner, 2010) and (Huang and Moe)). With few exceptions, factors that

potentiate P_i retention, such as low P_i -dietary intake or $1,25(OH)_2D$, lead to an increased expression of either all or some of these transporters. In contrast, factors that signal P_i loss, including PTH, dopamine or FGF-23, associate with reduced abundance of SLC34 proteins. Neither short term up- or down-regulation seems to involve changes on mRNA levels, suggesting a posttranscriptional control. The nature of this posttranscriptional mechanism has been clarified in the case of the PTH-induced downregulation of NaPi-IIa: PTH administration leads to phosphorylation of NHERF1, an adaptor protein that contributes to stabilize NaPi-IIa at the proximal BBM; phosphorylation of NHERF1 decreases its affinity for NaPi-IIa, resulting in a reduced stability and therefore removal of the cotransporter from the apical membrane (Deliot et al., 2005; Weinman et al., 2007). Whether or not similar protein-protein interaction mechanisms contribute to stabilize the membrane expression of NaPi-IIc and /or NaPi-IIb is unknown. K-deficiency and soluble Klotho are among the phosphaturic factors that do not act by reducing the expression of NaPi-IIa. Instead, K-deficiency in rats associates with higher NaPi-IIa (but reduced NaPi-IIc and PiT2); an altered lipid composition of the BBM has been postulated to inhibit NaPi-IIa activity in this metabolic situation (Breusegem et al., 2009; Zajicek et al., 2001). Soluble klotho has a phosphaturic effect independent of FGF23 that manifest prior to the reduction of NaPi-IIa abundance in BBM (Hu et al.); the precise mechanism remains unknown, although it appears to involve glycosylation of an unidentified factor (for review see (Huang and Moe)).

2.5.2 Pathophysiology and effect of naturally occurring mutations

Failure to maintain circulating levels of P_i constant results in pathological states associated with both hyper- and hypo-phosphatemia (for review see (Bergwitz and Juppner, 2010). Several heterozygous mutations of NaPi-IIa were reported in patients with nephrolithiasis and osteoporosis (Prie et al., 2002), but were not confirmed *in vitro* studies (Virkki et al., 2003). In addition, mutations of NaPi-IIa were identified in patients with autosomal recessive Fanconi syndrome and hypophosphatemic rickets (Magen et al.; Magen et al.), as well as in Sotos' patients exhibiting nephrocalcinosis and hypercalcemia but normal circulating P_i (Kenny et al.). In contrast, there is a large number of reported mutations of NaPi-IIc in patients with hypophosphatemic rickets with hypercalciuria (HHRH), e.g. (Bergwitz et al., 2006). These findings have challenged the assumption of NaPi-IIa being the major P_i-reabsorbing protein in the human kidney. For NaPi-IIb, several reports indicate that mutations of this transporter lead to alveolar and testicular microlithiasis ((Corut et al., 2006), for review see (Miyamoto et al.).

2.5.3 Pharmacology

Interest in inhibitors for SLC34 proteins focuses on blockers for NaPi-IIb as a means to reduce intestinal P_i absorption in chronic kidney disease. Transport by all SLC34 isoforms is blocked by the competitive inhibitor phosphonoformic acid or foscarnet (PFA) (Loghman-Adham, 1996). However, PFA has a high inhibition constant (~0.4-0.6 mM (e.g.(Villa-Bellosta and Sorribas, 2009) compared with the P_i apparent affinity and shows nephrotoxic effects. These features make it unattractive to limit dietary P_i absorption as a strategy to prevent hyperphosphatemia in cases where kidney function is compromised (Wallin and Ryrfeldt, 1995). Other pharmacologically interesting compounds with significantly lower

inhibition constants than PFA have been the subject of commercial studies (Weinstock, 2004). These possibly act in a non-competitive manner on NaPi-II proteins as reported for the compound JTP-59557 (Matsuo et al., 2005). A phosphophloretin compound was reported to exhibit inhibition at micromolar concentrations (Peerce et al., 2003) although its efficacy on heterologously expressed SLC34 proteins is unknown.

3. The SLC20 family (Type III Na^+-coupled P_i cotransporters, PiT-1, PiT-2)

3.1 PiT-1, PiT-2

The two known mammalian isoforms of SLC20 proteins (PiT-1, SLC20A1, and PiT-2, SLC20A2) were originally identified as retroviral receptors Glvr-1 (gibbon ape leukemia virus receptor) and Ram-1 (rat amphotropic leukemia virus receptor), respectively. Their role as Na^+-coupled P_i transporters was first reported in 1994 (Kavanaugh et al., 1994; Olah et al., 1994).

The mRNAs for both SLC20 isoforms are ubiquitously expressed in mice, yet cell specific localization of SLC20 proteins in different organs is still largely unknown (Collins et al., 2004). This widespread expression pattern is also reflected at the mRNA level of human tissue (Fig 1B) (Nishimura and Naito, 2008). In kidney, expression of both PiT1 and PiT-2 was detected, yet the cellular localization at the apical membrane of proximal tubular epithelia was described only for PiT-2 (Villa-Bellosta et al., 2009). In small intestine, expression both PiT-1 and PiT-2 has also been reported (Bai et al., 2000; Giral et al., 2009).

3.2 Kinetics and structure-function relationships of SLC20 proteins

3.2.1 Kinetics

The role of SLC20 proteins as Na^+-coupled P_i transporters was first confirmed by heterologous expression in *X. Laevis* oocytes and established that they were electrogenic and Na^+-dependent (Kavanaugh and Kabat, 1996; Kavanaugh et al., 1994). From these and other studies (e.g. (Bai et al., 2000; Bottger et al., 2006; Olah et al., 1994)) it became apparent that SLC20 proteins were functionally distinct from SLC34 proteins. These differences were highlighted in a detailed characterization of PiT-1 transport kinetics (Ravera et al., 2007). The most important kinetic property that distinguishes SLC20 from SLC34 proteins is that the former preferentially transport monovalent P_i with a 2:1 Na^+:P_i stoichiometry (Fig 2B) (Ravera et al., 2007; Saliba et al., 2006). Like all SLC34 proteins investigated to date, apparent affinities for P_i and Na^+ are typically d100 μM and ~50 mM respectively. PiT-1 is significantly less sensitive to pH compared to SLC34 proteins, whereby the maximum transport rate is relatively constant over a 3 pH units and the apparent P_i affinity decreases only at pH <6. The preference for monovalent P_i and insensitivity of transport kinetics to reduced pH means that SLC20 proteins can provide P_i transport capacity under conditions where SLC34 proteins are functionally compromised. Unlike SLC34 proteins that are exclusively Na^+ drive, for PiT-1, Li^+ can fully replace Na^+ as the driving cation, albeit with a significantly reduced transport rate (Ravera et al., 2007). Furthermore, in the absence of Na^+, lowering pH from 7.5 to 6.0 induces significant P_i uptake in *Xenopus* oocytes that expressed PiT-2, which suggest that H^+ may also substitute for Na^+ for this isoform (Bottger et al., 2006;

Villa-Bellosta et al., 2007). Na^+ is the first ion to interact followed by a random binding of Na^+ and P_i (Ravera et al., 2007). Finally, unlike SLC34 proteins, in which the final translocation of substrates is assumed electroneutral, the equivalent partial reaction may confer electrogenicity to PiT-1,2 (Virkki et al., 2007).

3.2.2 Structure-function relations.

The gene products of the SLC20 family belong to a unique class of proteins that are represented in all phyla (Bottger and Pedersen; Virkki et al., 2007). Compared with SLC34 proteins, relatively few structure-function studies have been undertaken. The current topology (Fig 2B) is proposed to comprise 12 TMDs with extracellular N- and C-terminal tails. This topology is suggested by bioinformatic predictions, tagging, cysteine scanning and *in vitro* glycosylation studies, e.g.(Bottger and Pedersen; Farrell et al., 2009; Salaun et al., 2001). Studies have also focused on identifying the viral receptor domains, which has led to further insight into the SLC20 protein structure-function relations (Bottger and Pedersen, 2004; Feldman et al., 2004; Salaun et al., 2001). For example, the large intracellular domain and associated TMDs have been removed and shown not to be required for retroviral recognition (Bottger and Pedersen, 2004), although it is unknown if the transport function was compromised. PiT-1 and PiT-2 differ in the location of their virus binding sites: for PiT-2 this has been identified in the 1st extracellular loop (Feldman et al., 2004), whereas that for PiT-1 has been suggested to be in the 4th extracellular loop (Johann et al., 1993) (Fig. 2B). Like SLC34, the structure of SLC20 proteins contains an inverted repeat architecture (Fig. 2B).

3.3 Physiological, pathophysiological and pharmaceutical aspects of SLC20 proteins

3.3.1 Physiological role and regulation

The ubiquitous expression of SLC20 protein at the mRNA level (Fig 1B) suggested they undertake a “housekeeping” role throughout the mammalian organism, however recent studies are bringing to light a diversity of more specific physiological roles. For example, PiT1 may play an important role in bone P_i homeostasis and its abundance is regulated by several factors involved in bone metabolism, including IGF-1 or BMP2 (reviewed in (Forster et al.; Miyamoto et al.). Furthermore, a number of studies also suggest a role of PiT-1 in hyperphosphatemia induced calcification of blood vessels (Lau et al.; Shanahan et al.) as well as in parathyroid function (Tatsumi et al., 1998). Recently a new function of PiT-1, independent of its transport function, has emerged. Depletion of PiT-1 expression in HeLa cells resulted in reduced cell proliferation and impaired mitosis (Beck et al., 2009). *In vivo* studies underscored these findings by showing that a PiT-1 knockout mouse is embryonic lethal, reflecting the importance of PiT-1 in liver development (Beck et al.), anemia and arrested growth (Festing et al., 2009).

With respect to the main organs for P_i homeostasis (Fig 1), the small intestine and kidney, the relative contributions of PiT-1/2 are largely unknown. In both organs PiT-1/2 may contribute only moderately (~5%) to transepithelial P_i transport (Forster et al.; Villa-Bellosta and Sorribas). In kidney, the apical abundance of PiT-2 is regulated by dietary intake of P_i (Villa-Bellosta et al., 2009), potassium deficiency (Breusegem et al., 2009) and PTH (Picard

et al., 2010); its regulation by phosphatonins remains to be investigated. In small intestine, the abundance of PiT-1 protein is altered by dietary P_i (Giral et al., 2009).

3.3.2 Pathophysiology and effect of naturally occurring mutations

Linkage analysis in a family with familial idiopathic basal ganglia calcification (Fahr disease) identified loss of function mutations in the SLC20A2 gene that represent the molecular basis for disturbed P_i homeostasis in the brain of these patients, implicating the involvement of PiT-2 in brain (Wang et al.)

3.3.3 Pharmacology

PFA is a weak inhibitor of PiT-1,2 mediated transport activity (Villa-Bellosta et al., 2007; Villa-Bellosta et al., 2009) and currently no specific inhibitors for SLC20 have been reported.

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Figure Legends

Fig 1.
A. The main organs involved in inorganic phosphate (P_i) homeostasis in man, indicating the principal sites of confirmed protein expression of members of the SLC20 and SLC34 families. Normally, dietary P_i input is balanced by excretion in feces and urine to maintain a reasonable stable circulating P_i pool. The kidney plays a crucial role in this balance by reabsorbing P_i from the glomerular filtrate.
B. The distribution of SLC34 (*upper panel*) and SLC20 (*lower panel*) at the mRNA level detected in human tissue. The high expression of both NaPi-IIa and NaPi-IIc in kidney, and NaPi-IIb in small intestine (indicated by arrows) is in stark comparison to the distribution of PiT-1, -2. NaPi-IIb mRNA is found in a number of other organs, but not the kidney. Data adapted from (Nishimura and Naito, 2008).

Fig 2
Cartoons illustrating the basic transport mechanism and molecular features of SLC34 (A) and SLC20 (B) proteins (adapted from (Ghezzi et al., 2009)). Topology models show the predicted transmembrane domains (numbered) and repeat regions (dark shading) for each protein family. The 3-D folding for proteins of both families is currently not known.

Tables

1. SLC34-Type II Na^+-phosphate cotransporter family

Human gene name	Protein name	Aliases	Predominant substrates	Transport type/coupling ions*	Tissue distribution and cellular/subcellular expression	Link to disease#	Human gene locus	Sequence accession ID	Splice variants and their features
SLC34A1	NaPi-IIa	Napi-3, NPT2, npt2	Inorganic phosphate (divalent)	C/Na/HPO_4^{2-}	Kidney (proximal tubule, apical membrane), osteoclasts, neurons	G/XLH, G/ADHR, A/OHO, nephrocalciosis.hypo-phosphatemia, urolithiasis, osteoporosis	5q35	NM003052.4	
SLC34A2	NaPi-IIb		Inorganic phosphate (divalent)	C/Na/HPO_4^{2-}	Small intestine, lung, testis, liver, secreting mammary gland	Pulmonary alveolar microlithiasis, testicular microlithiasis	4p15	NM006424.2	
SLC34A3	NaPi-IIc		Inorganic phosphate (divalent)	C/Na/HPO_4^{2-}	Kidney (proximal tubule, apical membrane)	Hypophosphatemic rickets with hypercalciuria	9q34	NM080877.2	
* C: cotransporter; E: exchanger; F: facilitated transporter; O: orphan transporter # A: acquired defect; G: genetic defect; P:pseudogene									

2. SLC20-Type III Na^+-phosphate cotransporter family

Human gene name	Protein name	Aliases	Predominant substrates	Transport type/coupling ions*	Tissue distribution and cellular/subcellular expression	Link to disease#	Human gene locus	Sequence accession ID	Splice variants and their features
SLC20A1	PiT-1	Gibbon ape leukemia virus receptor 1, GLVR1, Glvr1, FLJ41426, DKFZp686J2397	Inorganic phosphate (monovalent)	C/Na/$H_2PO_4^-$	Widely expressed		2q11-q14	NM005415	
SLC20A2	PiT-2	Amphotropic murine leukemia virus receptor 2, GLVR2, Glvr-2, MLVAR	Inorganic phosphate (monovalent)	C/Na/$H_2PO_4^-$	Widely expressed; kidney (proximal tubule, apical membrane)	G/Idiopathic basal ganglia calcification	8p12-p11	NM006749	
* C: cotransporter; E: exchanger; F: facilitated transporter; O: orphan transporter # A: acquired defect; G: genetic defect; P:pseudogene									

References

Andrini, O., Ghezzi, C., Murer, H., Forster, I.C., 2008. The leak mode of type II Na(+)-P(i) cotransporters. Channels (Austin) 2 (5), 346-357.
Andrini, O., Meinild, A.K., Ghezzi, C., Murer, H., Forster, I.C., Lithium interactions with Na+-coupled inorganic phosphate cotransporters: insights into the mechanism of sequential cation binding. Am J Physiol Cell Physiol 302 (3), C539-554.
Bacconi, A., Virkki, L.V., Biber, J., Murer, H., Forster, I.C., 2005. Renouncing electrogenicity is not free of charge: switching on electrogenicity in a Na^+-coupled phosphate cotransporter. Proceedings of the National Academy of Sciences of the United States of America 102, 12606-12611.
Bai, L., Collins, J.F., Ghishan, F.K., 2000. Cloning and characterization of a type III Na-dependent phosphate cotransporter from mouse intestine. Am J Physiol Cell Physiol 279 (4), C1135-1143.
Beck, L., Karaplis, A.C., Amizuka, N., Hewson, A.S., Ozawa, H., Tenenhouse, H.S., 1998. Targeted inactivation of Npt2 in mice leads to severe renal phosphate wasting, hypercalciuria, and skeletal abnormalities. Proceedings of the National Academy of Sciences of the United States of America 95 (9), 5372-5377.
Beck, L., Leroy, C., Beck-Cormier, S., Forand, A., Salaun, C., Paris, N., Bernier, A., Urena-Torres, P., Prie, D., Ollero, M., Coulombel, L., Friedlander, G., The phosphate transporter PiT1 (Slc20a1) revealed as a new essential gene for mouse liver development. PLoS One 5 (2), e9148.
Beck, L., Leroy, C., Salaun, C., Margall-Ducos, G., Desdouets, C., Friedlander, G., 2009. Identification of a novel function of PiT1 critical for cell proliferation and independent of its phosphate transport activity. J Biol Chem 284 (45), 31363-31374.
Bergwitz, C., Juppner, H., 2010. Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. Annu Rev Med 61, 91-104.
Bergwitz, C., Roslin, N.M., Tieder, M., Loredo-Osti, J.C., Bastepe, M., Abu-Zahra, H., Frappier, D., Burkett, K., Carpenter, T.O., Anderson, D., Garabedian, M., Sermet, I., Fujiwara, T.M., Morgan, K., Tenenhouse, H.S., Juppner, H., 2006. SLC34A3 Mutations in Patients with Hereditary Hypophosphatemic Rickets with Hypercalciuria Predict a Key Role for the Sodium-Phosphate Cotransporter NaPi-IIc in Maintaining Phosphate Homeostasis. Am J Hum Genet 78 (2), 179-192.
Bottger, P., Hede, S.E., Grunnet, M., Hoyer, B., Klaerke, D.A., Pedersen, L., 2006. Characterization of transport mechanisms and determinants critical for Na+-dependent Pi symport of the PiT family paralogs human PiT1 and PiT2. Am J Physiol Cell Physiol 291 (6), C1377-1387.
Bottger, P., Pedersen, L., Mapping of the minimal inorganic phosphate transporting unit of human PiT2 suggests a structure universal to PiT-related proteins from all kingdoms of life. BMC Biochem 12, 21.
Bottger, P., Pedersen, L., 2004. The central half of Pit2 is not required for its function as a retroviral receptor. J Virol 78 (17), 9564-9567.
Breusegem, S.Y., Takahashi, H., Giral-Arnal, H., Wang, X., Jiang, T., Verlander, J.W., Wilson, P., Miyazaki-Anzai, S., Sutherland, E., Caldas, Y., Blaine, J.T., Segawa, H., Miyamoto, K., Barry, N.P., Levi, M., 2009. Differential regulation of the renal sodium-phosphate cotransporters NaPi-IIa, NaPi-IIc, and PiT-2 in dietary potassium deficiency. Am J Physiol Renal Physiol 297 (2), F350-361.
Collins, J.F., Bai, L., Ghishan, F.K., 2004. The SLC20 family of proteins: dual functions as sodium-phosphate cotransporters and viral receptors. Pflugers Archiv - European Journal of Physiology 447 (5), 647-652.
Corut, A., Senyigit, A., Ugur, S.A., Altin, S., Ozcelik, U., Calisir, H., Yildirim, Z., Gocmen, A., Tolun, A., 2006. Mutations in SLC34A2 cause pulmonary alveolar microlithiasis and are possibly associated with testicular microlithiasis. Am J Hum Genet 79 (4), 650-656.
Custer M, L.M., Biber J, Murer H, Kaisslling 1994. Expression of Na/Pi cotransport in rat kidney: Localization by RT-PCR and immunohistochemistry. Am J Physiol Renal Physiol 266, F767-F774
Deliot, N., Hernando, N., Horst-Liu, Z., Gisler, S.M., Capuano, P., Wagner, C.A., Bacic, D., O'Brien, S., Biber, J., Murer, H., 2005. Parathyroid hormone treatment induces dissociation of type IIa Na+-P(i) cotransporter-Na+/H+ exchanger regulatory factor-1 complexes. Am J Physiol Cell Physiol 289 (1), C159-167.
Farrell, K.B., Tusnady, G.E., Eiden, M.V., 2009. New structural arrangement of the extracellular regions of the phosphate transporter SLC20A1, the receptor for gibbon ape leukemia virus. J Biol Chem 284 (43), 29979-29987.
Feldman, S.A., Farrell, K.B., Murthy, R.K., Russ, J.L., Eiden, M.V., 2004. Identification of an extracellular domain within the human PiT2 receptor that is required for amphotropic murine leukemia virus binding. J Virol 78 (2), 595-602.
Festing, M.H., Speer, M.Y., Yang, H.Y., Giachelli, C.M., 2009. Generation of mouse conditional and null alleles of the type III sodium-dependent phosphate cotransporter PiT-1. Genesis 47 (12), 858-863.
Forrest, L.R., Kramer, R., Ziegler, C., The structural basis of secondary active transport mechanisms. Biochim Biophys Acta 1807 (2), 167-188.

Forster, I., Hernando, N., Sorribas, V., Werner, A., Phosphate transporters in renal, gastrointestinal, and other tissues. Adv Chronic Kidney Dis 18 (2), 63-76.
Forster, I.C., Biber, J., Murer, H., 2000. Proton-sensitive transitions of renal type II Na^+-coupled phosphate cotransporter kinetics. Biophysical Journal 79 (1), 215-230.
Forster, I.C., Hernando, N., Biber, J., Murer, H., 2006. Proximal tubular handling of phosphate: A molecular perspective. Kidney Int 70 (9), 1548-1559.
Forster, I.C., Kohler, K., Biber, J., Murer, H., 2002. Forging the link between structure and function of electrogenic cotransporters: the renal type IIa Na^+/P_i cotransporter as a case study. Progress in Biophysics and Molecular Biology 80 (3), 69-108.
Forster, I.C., Loo, D.D., Eskandari, S., 1999. Stoichiometry and Na^+ binding cooperativity of rat and flounder renal type II Na^+-P_i cotransporters. American Journal of Physiology 276 (4 Pt 2), F644-649.
Ghezzi, C., Murer, H., Forster, I.C., 2009. Substrate interactions of the electroneutral Na+-coupled inorganic phosphate cotransporter (NaPi-IIc). J Physiol 587 (Pt 17), 4293-4307.
Giral, H., Caldas, Y., Sutherland, E., Wilson, P., Breusegem, S., Barry, N., Blaine, J., Jiang, T., Wang, X.X., Levi, M., 2009. Regulation of rat intestinal Na-dependent phosphate transporters by dietary phosphate. Am J Physiol Renal Physiol 297 (5), F1466-1475.
Gisler, S.M., Fuster, D., Radanovic, T., Hall, R.A., Engles, K., Stagljar, I., Murer, H., Biber, J., Moe, O.W., 2007. Application of the type II split-ubiquitin membrane yeast two-hybrid system using whole renal epithelial transmembrane proteins as baits. Biochemistry submitted?
Hernando, N., Karim-Jimenez, Z., Biber, J., Murer, H., 2001. Molecular determinants for apical expression and regulatory membrane retrieval of the type IIa Na/Pi cotransporter. Kidney Int 60 (2), 431-435.
Hilfiker, H., Hattenhauer, O., Traebert, M., Forster, I., Murer, H., Biber, J., 1998. Characterization of a murine type II sodium-phosphate cotransporter expressed in mammalian small intestine. Proceedings of the National Academy of Sciences of the United States of America 95 (24), 14564-14569.
Hu, M.C., Shi, M., Zhang, J., Pastor, J., Nakatani, T., Lanske, B., Razzaque, M.S., Rosenblatt, K.P., Baum, M.G., Kuro-o, M., Moe, O.W., Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. FASEB J 24 (9), 3438-3450.
Huang, C.L., Moe, O.W., Klotho: a novel regulator of calcium and phosphorus homeostasis. Pflugers Arch 462 (2), 185-193.
Jaureguiberry, G., Carpenter, T.O., Forman, S., Juppner, H., Bergwitz, C., 2008. A novel missense mutation in SLC34A3 that causes hereditary hypophosphatemic rickets with hypercalciuria in humans identifies threonine 137 as an important determinant of sodium-phosphate cotransport in NaPi-IIc. Am J Physiol Renal Physiol 295 (2), F371-379.
Johann, S.V., van Zeijl, M., Cekleniak, J., O'Hara, B., 1993. Definition of a domain of GLVR1 which is necessary for infection by gibbon ape leukemia virus and which is highly polymorphic between species. J Virol 67 (11), 6733-6736.
Karim-Jimenez, Z., Hernando, N., Biber, J., Murer, H., 2000. A dibasic motif involved in parathyroid hormone-induced down-regulation of the type IIa NaPi cotransporter. Proc Natl Acad Sci U S A 97 (23), 12896-12901.
Karim-Jimenez, Z., Hernando, N., Biber, J., Murer, H., 2001. Molecular determinants for apical expression of the renal type IIa Na+/Pi-cotransporter. Pflugers Arch 442 (5), 782-790.
Kavanaugh, M.P., Kabat, D., 1996. Identification and characterization of a widely expressed phosphate transporter/retrovirus receptor family. Kidney International 49 (4), 959-963.
Kavanaugh, M.P., Miller, D.G., Zhang, W., Law, W., Kozak, S.L., Kabat, D., Miller, A.D., 1994. Cell-surface receptors for gibbon ape leukemia virus and amphotropic murine retrovirus are inducible sodium-dependent phosphate symporters. Proceedings of the National Academy of Sciences of the United States of America 91 (15), 7071-7075.
Kenny, J., Lees, M.M., Drury, S., Barnicoat, A., Van't Hoff, W., Palmer, R., Morrogh, D., Waters, J.J., Lench, N.J., Bockenhauer, D., Sotos syndrome, infantile hypercalcemia, and nephrocalcinosis: a contiguous gene syndrome. Pediatr Nephrol 26 (8), 1331-1334.
Kohler, K., Forster, I.C., Lambert, G., Biber, J., Murer, H., 2000. The functional unit of the renal type IIa Na+/Pi cotransporter is a monomer. Journal of Biological Chemistry 275 (34), 26113-26120.
Lau, W.L., Festing, M.H., Giachelli, C.M., Phosphate and vascular calcification: Emerging role of the sodium-dependent phosphate co-transporter PiT-1. Thromb Haemost 104 (3), 464-470.
Loghman-Adham, M., 1996. Use of phosphonocarboxylic acids as inhibitors of sodium-phosphate cotransport. General Pharmacology 27 (2), 305-312.
Magagnin, S., Werner, A., Markovich, D., Sorribas, V., Stange, G., Biber, J., Murer, H., 1993. Expression cloning of human and rat renal cortex Na/P_i cotransport. Proceedings of the National Academy of Sciences of the United States of America 90 (13), 5979-5983.

Magen, D., Berger, L., Coady, M.J., Ilivitzki, A., Militianu, D., Tieder, M., Selig, S., Lapointe, J.Y., Zelikovic, I., Skorecki, K., A loss-of-function mutation in NaPi-IIa and renal Fanconi's syndrome. N Engl J Med 362 (12), 1102-1109.
Magen, D., Zelikovic, I., Skorecki, K., Genetic disorders of renal phosphate transport. N Engl J Med 363 (18), 1774; author reply 1774-1775.
Matsuo, A.Y., Duarte, R.M., Val, A.L., 2005. Unidirectional sodium fluxes and gill CYP1A induction in an Amazonian fish (Hyphessobrycon erythrostigma) exposed to a surfactant and to crude oil. Bull Environ Contam Toxicol 75 (5), 851-858.
Miyamoto, K., Haito-Sugino, S., Kuwahara, S., Ohi, A., Nomura, K., Ito, M., Kuwahata, M., Kido, S., Tatsumi, S., Kaneko, I., Segawa, H., Sodium-dependent phosphate cotransporters: lessons from gene knockout and mutation studies. J Pharm Sci 100 (9), 3719-3730.
Nishimura, M., Naito, S., 2008. Tissue-specific mRNA expression profiles of human solute carrier transporter superfamilies. Drug Metab Pharmacokinet 23 (1), 22-44.
Olah, Z., Lehel, C., Anderson, W.B., Eiden, M.V., Wilson, C.A., 1994. The cellular receptor for gibbon ape leukemia virus is a novel high affinity sodium-dependent phosphate transporter. Journal of Biological Chemistry 269 (41), 25426-25431.
Peerce, B.E., Fleming, R.Y., Clarke, R.D., 2003. Inhibition of human intestinal brush border membrane vesicle Na+-dependent phosphate uptake by phosphophloretin derivatives. Biochem Biophys Res Commun 301 (1), 8-12.
Picard, N., Capuano, P., Stange, G., Mihailova, M., Kaissling, B., Murer, H., Biber, J., Wagner, C.A., 2010. Acute parathyroid hormone differentially regulates renal brush border membrane phosphate cotransporters. Pflugers Arch 460 (3), 677-687.
Prie, D., Huart, V., Bakouh, N., Planelles, G., Dellis, O., Gerard, B., Hulin, P., Benque-Blanchet, F., Silve, C., Grandchamp, B., Friedlander, G., 2002. Nephrolithiasis and osteoporosis associated with hypophosphatemia caused by mutations in the type 2a sodium-phosphate cotransporter.[comment]. New England Journal of Medicine 347 (13), 983-991.
Radanovic, T., Wagner, C.A., Murer, H., Biber, J., 2005. Regulation of intestinal phosphate transport. I. Segmental expression and adaptation to low-P_i diet of the type IIb Na^+-P_i cotransporter in mouse small intestine. American Journal of Physiology - Gastrointestinal & Liver Physiology 288 (3), G496-500.
Ravera, S., Virkki, L.V., Murer, H., Forster, I.C., 2007. Deciphering PiT transport kinetics and substrate specificity using electrophysiology and flux measurements. American Journal of Physiology-Cell Physiology
Sabbagh, Y., O'Brien, S.P., Song, W., Boulanger, J.H., Stockmann, A., Arbeeny, C., Schiavi, S.C., 2009. Intestinal npt2b plays a major role in phosphate absorption and homeostasis. J Am Soc Nephrol 20 (11), 2348-2358.
Salaun, C., Rodrigues, P., Heard, J.M., 2001. Transmembrane topology of PiT-2, a phosphate transporter-retrovirus receptor. J Virol 75 (12), 5584-5592.
Saliba, K.J., Martin, R.E., Broer, A., Henry, R.I., McCarthy, C.S., Downie, M.J., Allen, R.J., Mullin, K.A., McFadden, G.I., Broer, S., Kirk, K., 2006. Sodium-dependent uptake of inorganic phosphate by the intracellular malaria parasite. Nature 443 (7111), 582-585.
Screpanti, E., Hunte, C., 2007. Discontinuous membrane helices in transport proteins and their correlation with function. J Struct Biol 159 (2), 261-267.
Segawa, H., Kaneko, I., Takahashi, A., Kuwahata, M., Ito, M., Ohkido, I., Tatsumi, S., Miyamoto, K., 2002. Growth-related renal type II Na/Pi cotransporter. Journal of Biological Chemistry 277 (22), 19665-19672.
Segawa, H., Onitsuka, A., Kuwahata, M., Hanabusa, E., Furutani, J., Kaneko, I., Tomoe, Y., Aranami, F., Matsumoto, N., Ito, M., Matsumoto, M., Li, M., Amizuka, N., Miyamoto, K., 2009. Type IIc sodium-dependent phosphate transporter regulates calcium metabolism. J Am Soc Nephrol 20 (1), 104-113.
Shanahan, C.M., Crouthamel, M.H., Kapustin, A., Giachelli, C.M., Arterial calcification in chronic kidney disease: key roles for calcium and phosphate. Circ Res 109 (6), 697-711.
Shibasaki, Y., Etoh, N., Hayasaka, M., Takahashi, M.O., Kakitani, M., Yamashita, T., Tomizuka, K., Hanaoka, K., 2009. Targeted deletion of the tybe IIb Na(+)-dependent Pi-co-transporter, NaPi-IIb, results in early embryonic lethality. Biochem Biophys Res Commun 381 (4), 482-486.
Tatsumi, S., Segawa, H., Morita, K., Haga, H., Kouda, T., Yamamoto, H., Inoue, Y., Nii, T., Katai, K., Taketani, Y., Miyamoto, K.I., Takeda, E., 1998. Molecular cloning and hormonal regulation of PiT-1, a sodium-dependent phosphate cotransporter from rat parathyroid glands. Endocrinology 139 (4), 1692-1699.
Villa-Bellosta, R., Bogaert, Y.E., Levi, M., Sorribas, V., 2007. Characterization of phosphate transport in rat vascular smooth muscle cells. Implications for vascular calcification. Arterioscler Thromb Vasc Biol.
Villa-Bellosta, R., Ravera, S., Sorribas, V., Stange, G., Levi, M., Murer, H., Biber, J., Forster, I.C., 2009. The Na+-Pi cotransporter PiT-2 (SLC20A2) is expressed in the apical membrane of rat renal proximal tubules and regulated by dietary Pi. Am J Physiol Renal Physiol 296 (4), F691-699.

Villa-Bellosta, R., Sorribas, V., Compensatory regulation of the sodium/phosphate cotransporters NaPi-IIc (SCL34A3) and Pit-2 (SLC20A2) during Pi deprivation and acidosis. Pflugers Arch 459 (3), 499-508.
Villa-Bellosta, R., Sorribas, V., 2009. Different effects of arsenate and phosphonoformate on P(i) transport adaptation in opossum kidney cells. Am J Physiol Cell Physiol 297 (3), C516-525.
Virkki, L.V., Biber, J., Murer, H., Forster, I.C., 2007. Phosphate transporters: a tale of two solute carrier families. Am J Physiol Renal Physiol 293 (3), F643-654.
Virkki, L.V., Forster, I.C., Hernando, N., Biber, J., Murer, H., 2003. Functional characterization of two naturally occurring mutations in the human sodium-phosphate cotransporter type IIa. Journal of Bone and Mineral Research 18 (12), 2135-2141.
Virkki, L.V., Murer, H., Forster, I.C., 2006. Voltage clamp fluorometric measurements on a type II Na^+-coupled P_i cotransporter: shedding light on substrate binding order. Journal of General Physiology 127, 539-555.
Wallin, A., Ryrfeldt, A., 1995. The toxicity of Foscarnet (phosphonoformic acid, trisodium salt; PFA) studied in cultured dog renal Tubular Cells. Toxicol In Vitro 9 (3), 237-244.
Wang, C., Li, Y., Shi, L., Ren, J., Patti, M., Wang, T., de Oliveira, J.R., Sobrido, M.J., Quintans, B., Baquero, M., Cui, X., Zhang, X.Y., Wang, L., Xu, H., Wang, J., Yao, J., Dai, X., Liu, J., Zhang, L., Ma, H., Gao, Y., Ma, X., Feng, S., Liu, M., Wang, Q.K., Forster, I.C., Zhang, X., Liu, J.Y., Mutations in SLC20A2 link familial idiopathic basal ganglia calcification with phosphate homeostasis. Nat Genet.
Weinman, E.J., Biswas, R.S., Peng, G., Shen, L., Turner, C.L., E, X., Steplock, D., Shenolikar, S., Cunningham, R., 2007. Parathyroid hormone inhibits renal phosphate transport by phosphorylation of serine 77 of sodium-hydrogen exchanger regulatory factor-1. J Clin Invest 117 (11), 3412-3420.
Weinstock, J., 2004. Inhibitors of sodium-dependent phosphate transport. Expert Opinion Therapeutic Patents 14 (1), 3.
Werner, A., Kinne, R.K., 2001. Evolution of the Na-P_i cotransport systems. American Journal of Physiology 280 (2), R301-312.
Zajicek, H.K., Wang, H., Puttaparthi, K., Halaihel, N., Markovich, D., Shayman, J., Beliveau, R., Wilson, P., Rogers, T., Levi, M., 2001. Glycosphingolipids modulate renal phosphate transport in potassium deficiency. Kidney Int 60 (2), 694-704.